Remarks

Claims 1-13 were pending in the subject application. By this Amendment, the applicants have amended claim 1 and cancelled claims 2 and 6. No new matter has been added by these amendments. Accordingly, claims 1, 3-5, and 7-13 are now before the Examiner for further consideration.

The amendments to the claims have been made in an effort to lend greater clarity to the claimed subject matter and to expedite prosecution. The amendments should not be taken to indicate the applicants' agreement with, or acquiescence to, the rejections of record. Favorable consideration of the claims now presented, in view of the remarks and amendments set forth herein, is earnestly solicited.

Claims 1-13 have been rejected under 35 U.S.C. §102(b) as being anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over RO-115,885 (hereinafter the RO '885 reference). The applicants respectfully traverse this ground for rejection because the cited reference does not teach or suggest the applicants' advantageous method for preparing a metal salt of a medium chain fatty acid.

Since this rejection is listed in the alternative, the applicants will address the issues of anticipation and obviousness separately. Turning first to the rejection of claims 1-13 under 35 U.S.C. §102(b) as being anticipated by the RO '885 reference, the applicants respectfully submit that the claimed invention, as currently presented, is clearly distinguishable from the method of the RO '885 reference.

By this Amendment, claim 1 has been amended to recite that the metal salt that is reacted with the free fatty acid comprises "at least one metal bicarbonate or metal carbonate." The RO '885 reference, on the other hand, fails to disclose this novel and advantageous feature required by the claimed invention. Instead, the only salts taught by the RO '885 reference are sodium hydroxide and potassium hydroxide, each of which is clearly not a metal bicarbonate or metal carbonate.

Moreover, the claimed invention is drawn to a method of preparing a metal salt of a medium-chain fatty acid, including solubilizing a free fatty acid that has a chain length of from six to twelve carbons. The RO '885 reference fails to disclose any free fatty acids, but the Action cites the Bailey reference (Bailey's Industrial Oil and Fat Products, Vol. 2, Edible Oil and Fat Products: Oils and

Oilseeds, 1996) for the proposition that a minimal amount (0.3-1.8%) of free fatty acids would be present in canola, rapeseed, or soybean oil. However, it is known in the art that these oils do not contain medium-chain fatty acids but instead include a mixture of long-chain length fatty acids, the shortest of these containing sixteen carbon atoms (e.g. palmitic acid). As support for this proposition, the applicants have attached hereto scientific articles by Kurata *et al.* (Rapid Discrimination of Fatty Acid Composition..., Analytical Sciences 21, 1457-1465, 2005) and Pospisil *et al.* (Fatty Acid Composition..., Agriculture Conspectus Scientificus 72, 187-193, 2007). As evidenced by the Kurata *et al.* and Pospisil *et al.* articles, the oils that may be present in the starting materials of the RO '885 reference would not include any free fatty acids with a chain length of six to twelve carbons, as required by the present invention (see, e.g., Table 1 of Kurata *et al.*). Instead, the shortest free fatty acid that may be present in the RO '885 reference would have at least sixteen carbon atoms, well in excess of the maximum of twelve recited in the claims of the subject invention.

It is a basic premise of patent law that in order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 U.S.P.Q.2d 1001, 1010 (Fed. Cir. 1991). As discussed above, the RO '885 reference fails to teach certain aspects of the claimed invention. Specifically, there is no disclosure of reacting a free fatty acid with a metal salt, wherein the metal salt comprises at least one metal bicarbonate or metal carbonate. The RO '885 reference also fails to disclose solubilizing a free fatty acid that has a chain length of from six to twelve carbons.

Accordingly, reconsideration and withdrawal of the rejection under §102(b) based on the RO '885 reference is respectfully requested.

Turning now to the rejection of claims 1-13 under 35 U.S.C. §103(a) as being obvious over the RO '885 reference, the applicants respectfully submit that the RO '885 reference does not teach or suggest the claimed invention.

The RO '885 reference discloses a process for producing soap by subjecting the triglycerides present in animal fats or vegetable oils to metholysis and saponification by treatment with sodium or potassium hydroxide. There is no disclosure anywhere in the RO '885 reference of reacting any fatty

acids with anything, let alone reacting at least one fatty acid with a metal salt, as required by the claimed invention.

As the Examiner is aware, all the claim limitations must be taught or suggested by the prior art in order to establish a *prima facie* case of obviousness of a claimed invention (*CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) citing *In re Royka*, 490 F.2d 981, 985 (C.C.P.A. 1974)). The Action indicates that there would be a small amount of free fatty acids present in the starting materials of the RO '885 reference and that these would inherently react with the hydroxide salt. The Bailey reference is cited by the Examiner for the proposition that a minimal amount (0.3-1.8%) of free fatty acids would be present in canola, rapeseed, or soybean oil.

The Action asserts at page 3 that this minimal amount of free fatty acids "will react with the methanol and sodium hydroxide to form the fatty acid salts independent of the triglycerides being transesterified and then saponified" (emphasis added). However, "[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." *Ex parte Levy,* 17 USPQ2d 1461, 1464 (BPAI 1990) (emphasis in original). Here, the Examiner has failed to provide such a technical reasoning as to why such a minimal amount of free fatty acids would inherently react with methanol and sodium hydroxide despite the fact that the other reactions are taking place. The applicants submit that free fatty acids that may be present in the starting materials of the RO '885 process would not necessarily react with the sodium hydroxide. As the primary (and intended) reactions of the triglycerides are taking place, using reactants and creating products, it is by no means certain that free fatty acids present in an amount less than 2% would inherently react with sodium hydroxide to produce a fatty acid salt.

Furthermore, as discussed above and demonstrated by the attached Kurata *et al.* and Pospisil *et al.* articles, the majority of known vegetable and animal oils, including canola, rapeseed, and soybean oil, do not contain medium-chain length fatty acids. Instead, these oils only have long-chain length fatty acids, with the shortest having sixteen carbon atoms, which is significantly more than the six to twelve carbon atoms required by the claimed invention. Due to differences in the physical chemistry of medium-chain length and long-chain length fatty acids (e.g. water-solubility, lipophilicity, acid strength as reflected by the acid dissociation constant of the carboxyl group), art

pertaining to the preparation of metal salts of long-chain length fatty acids is unlikely to even be relevant to the preparation of metal salts of medium-chain length fatty acids.

Thus, even if it is assumed, for the sake of argument, that long-chain free fatty acids are present in the starting materials of the RO '885 reference and that they would react with sodium hydroxide to produce a fatty acid salt, this reference still fails to teach or suggest a method for preparing a metal salt of a medium-chain fatty acid, as in the present invention.

Also, a skilled artisan would not have found a reason to modify the RO '885 reference to include a free fatty acid with a shorter chain length because the RO '885 reference does not even disclose the presence of any free fatty acids; rather, this is considered by the Examiner to be an inherent property. In addition, even if a skilled artisan had been aware of the presence of any long-chain length free fatty acids in the starting materials, the RO '885 reference details a process for producing soap by subjecting the triglycerides present in animal fats or vegetable oils to metholysis and saponification by treatment with sodium or potassium hydroxide. There is no disclosure anywhere in the RO '885 reference of reacting any fatty acids with anything, so one of ordinary skill in the art would not have been motivated to modify any free fatty acids that may be present in the starting materials of the RO '855 reference.

Moreover, assuming once again, for the sake of argument, that free fatty acids are present in the starting materials and that they would react with sodium hydroxide to produce a fatty acid salt, there is still no teaching or suggestion in the RO '885 reference of the use of a metal salt comprising at least one metal bicarbonate or metal carbonate. The applicants note that this limitation, now present in claim 1, was previously recited in claim 6. Though claim 6 was included in the rejection based on the RO '885 reference, no reason was given in the Action as to why the use of a metal salt comprising at least one metal bicarbonate or metal carbonate would have been obvious.

The Federal Circuit has stated that "rejections on obviousness cannot be sustained with mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *In re Kahn*, 441 F.3d 977, 988, 78 USPQ2d 1329, 1336 (Fed. Cir. 2006). Moreover, to establish a *prima facie* case of obviousness, the Examiner must show that "there was an apparent reason to combine the known elements in the

fashion claimed" by the applicants. *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, 127 S. Ct. 1727, 82 U.S.P.Q.2d 1385 (2007); MPEP §2142.

As discussed above, the methods of the RO '885 reference are directed to the production of soaps from animal fats and vegetable oils. A skilled artisan would have had no reason to modify the RO '885 process to react free fatty acids with a metal salt comprising at least one metal bicarbonate or metal carbonate, especially considering the fact that there is no explicit disclosure in the RO '885 reference of the presence of free fatty acids. In fact, this modification would be contrary to the purpose of the RO '885 reference of breaking down animal fats and vegetable oils to make soaps. If a proposed modification would render the (purported) prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. §2143.01(V); *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

Furthermore, "[i]n determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." MPEP §2141.02 (citing *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983)).

In this case, one of ordinary skill in the art would not have had a reason to substitute any other salts for the hydroxide salts disclosed. It is important to note once again that there is no teaching in the RO '885 reference of the reaction of any free fatty acids; rather, the Action asserts that this is an inherent property. However, a skilled artisan would not have found a reason to use any other salt, such as a metal salt comprising at least one metal bicarbonate or metal carbonate, that could react with free fatty acids that may be present in a minimal amount, especially since there is no explicit disclosure of the presence of any free fatty acids in the RO '885 reference.

Instead, only the applicants' own disclosure provides a reason to react a free fatty acid with a metal salt comprising at least one metal bicarbonate or metal carbonate. It is impermissible to use the claimed invention as an instruction manual or "template" to piece together the teachings of the prior art so that the claimed invention is rendered obvious. The Court of Appeals for the Federal Circuit has stated that "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir. 1992).

As discussed above, the claimed element of reacting a free fatty acid with at least one metal salt would not necessarily be present in the RO '885 reference. However, even if it is assumed, for the sake of argument, that this reaction would inherently occur, there is still no disclosure or suggestion of the use of a metal salt comprising at least one metal bicarbonate or metal carbonate. There is also no teaching of solubilizing a free fatty acid that has a chain length of from six to twelve carbons. Additionally, a skilled artisan would have no reason to modify the RO '885 reference to include these advantageous features of the claimed invention.

Accordingly, the applicants respectfully request reconsideration and withdrawal of the rejection under §103 based on the RO '885 reference.

Claims 1, 3, 5, 6, 11, and 12 have been rejected under 35 U.S.C. §102(b) as being anticipated by Rieber *et al.* (U.S. Patent No. 4,235,794). The applicants respectfully traverse this ground for rejection.

By this Amendment, the applicants have amended claim 1 to recite that the solvent in which the at least one free fatty acid is solubilized comprises an alcohol. Rieber *et al.*, on the other hand, fail to disclose the use of a solvent comprising an alcohol, as required by the claimed invention. Instead, the Rieber *et al.* reference describes a method for the manufacture of metal soap using only water or water-based suspensions to form granulates of the metal soaps. In fact, the word "alcohol" does not even appear anywhere in the text of Rieber *et al.*

Furthermore, Rieber *et al.* disclose reacting free fatty acids with a metal salt in the presence of a solvent. Exemplification is provided by the synthesis of calcium caprate and cadmium laurate whereby capric acid and lauric acid are suspended in water and reacted with their respective metal hydroxides. Thus, Rieber *et* al. disclose reaction of a water insoluble fatty acid (see, e.g., claim 1 of Rieber *et al.*). By contrast, the present invention requires "solubilizing at least one free fatty acid in solvent." In other words, Rieber *et al.* disclose the synthesis of an impure fatty acid salt by reaction of an insoluble fatty acid reactant in the presence of water to yield an insoluble (precipitated) fatty acid metal salt product contaminated with unreacted fatty acid (see examples 1-29) and hydroxide base, whereas the present invention teaches the synthesis of a high purity medium-chain length fatty acid salt by reaction of solubilized fatty acid reactant in a non-aqueous solvent to yield either a water-soluble (e.g. alkali metal) salts or water insoluble (e.g. alkaline earth metal) salts. Rieber *et al.*

do not disclose the synthesis of water-soluble (e.g. sodium, potassium) alkali metal salts of medium-chain length fatty acids.

In fact, as one of ordinary skill in the art would recognize, attempting to synthesize water-soluble sodium caprate by following the procedure described in Rieber *et al.*, wherein calcium hydroxide is replaced by sodium carbonate, would fail to give any product. Similarly, the calcium caprate product obtained by following the protocol described Rieber *et al.* is contaminated with unreacted base which co-precipitates with the product. On the other hand, preparation of calcium caprate following the procedure of the subject invention gives a high purity product facilitated by washing of the insoluble salt with ethanol followed by tert-butyl methyl ether (e.g. to remove impurities).

As discussed above, in order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic, supra.* Rieber *et al.* do not teach solubilizing at least one free fatty acid in solvent, wherein the solvent comprises an alcohol. This novel and advantageous feature is required by the claimed invention.

Additionally, the applicants note that this rejection under §102(b) based on Rieber *et al.* was not applied to claim 2, as previously presented. Claim 2 included the limitation, now present in amended claim 1, that the solvent comprises an alcohol.

Accordingly, the applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §102(b) based on Rieber *et al.*

In view of the foregoing remarks and the amendments to the claims, the applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

The applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

David Saliwanchik

David R. Saliwanchik
Patent Attorney
Registration No. 31,794
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

DRS/la

Attachments: Kurata *et al.* article
Pospisil *et al.* article

Fatty Acid Composition in Oil of Recent Rapeseed Hybrids and 00-Cultivars

Milan POSPIŠIL [1](✉), Dubravka ŠKEVIN [2], Zvonko MUSTAPIĆ [1], Sandra NEĐERAL NAKIĆ [2], Jasminka BUTORAC [1], Dunja MATIJEVIĆ [1]

Summary

Fatty acid composition in oil of seven new hybrids ('Artus', 'Baldur', 'Exact', 'Executive', 'Extra', 'RG 9908', 'RG 9909') and eight 00-cultivars ('Aviso', 'Bristol', 'Canary', 'Dexter', 'Ella', 'Kosto', 'Navajo', 'Royal') of rapeseed was investigated in the period 2003-2005. The experiments were placed in the experimental field of the Faculty of Agriculture in Zagreb. Fatty acid composition was determined by gas chromatography of their methyl esters, and the oil iodine number was calculated as well. The studied new rapeseed hybrids and 00-cultivars contained no erucic acid or it was present far below 2%. The average content of oleic acid was 61.88±2.64% in hybrids and 62.54±3.90% in 00-cultivars, the content of linoleic acid was 20.52±1.49% and 19.57±2.51%, the content of linolenic acid was 8.39±1.50% and 7.92±2.12%, the content of palmitic acid was 5.13±0.48% and 5.50±0.51%, and the content of stearic acid was 1.48±0.16% and 1.58±0.19%, respectively. This ratio of fatty acids confirms the high nutritive quality of rapeseed oil. The iodine value was 112±2 in oil from hybrids and 110±4 in oil from 00-cultivars.

In both investigated groups there were no differences in fatty acid composition which could influence the quality and stability of rapeseed oil. The average values in oils obtained from hybrids as well as from 00-cultivars are inside the data prescribed in law regulations. Although, there were several samples in which oleic and palmitic acid contents were above and linoleic and linolenic acid contents (as well as the iodine values) below the limit values, what ought to be incorporated into the revision of present regulations on vegetable oils.

Fatty acid composition in hybrids and in 00-cultivars was greatly influenced by weather conditions. In the year with higher mean monthly air temperatures and less precipitation during May and June compared to average long-term weather conditions for these months, oil contained more oleic and less linoleic and linolenic acid.

Key words

rapeseed, hybrids, 00-cultivars, fatty acid composition

[1] University of Zagreb, Faculty of Agriculture, Department of Field Crop Production, Svetošimunska 25, 10000 Zagreb, Croatia
✉ e-mail: mpospisil@agr.hr
[2] University of Zagreb, Faculty of Food Technology and Biotechnology, Laboratory for Oil and Fat Technology, Pierottijeva 6, 10000 Zagreb, Croatia

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Introduction

Rapeseed can be cultivated in the cooler agricultural regions and as a winter crop in temperate zones, producing at least 40% of oil and a meal containing 38–40% of high quality protein. It is now number three, after soybean oil and palm oil, in ranking of the 17 commodity oils and fats reported by Oil World. It has attained this position after rapid growth during the last twenty years and further increases expected in the next twenty years should allow it to maintain its market share of 13-14% (Gunstone, 2001). There is much interest in the potential of rapeseed oil in human and animal nutrition and in its industrial applications. Some nutritionists believe that it is almost ideal to replace traditional predominantly saturated fats in the "Western" diet. The residual high protein meal is used mainly for animal feed. Petrochemical industrialists may see much potential in rapeseed oil as a source of an environmental friendly biodiesel as well as in production of non-food products such as surfactants, plastic additives and lubricants.

Rapeseed plants have been the subject of intensive breeding projects in the last fifteen years, which include modifications in the fatty acid composition of the oil. A range of cultivars was created whose oils have a modified fatty acid profile in comparison with the conventional cultivars. The main focus was on the content of erucic acid, of oleic acid and of medium-chain fatty acids (Töpfer and Friedt, 1999; Piazza and Foglia, 2001) as well as a meal with very low levels of sulfur-containing glucosinolates. Development and introduction of new rapeseed cultivars and restaurated hybrids into exploitation, as well as the improvement of oil and meal quality influenced the extension of this crop, especially in Europe.

The results in the previous research of rapeseed cultivation in Croatia follow the European trends: instead of eruca acid, the dominating fatty acid in triglycerides is oleic acid (over 60%), and they contain the reduced content of linolenic acid (under 10%) and increased content of linoleic acid (10–20%) (Mustapić and Pospišil, 1995; Pospišil et al., 2000). Besides, the oil of new cultivars is also suitable for biodiesel production and the meal contains the low amount of glucosinolates. Rapeseed hybrids are successfully grown in many countries of western and northern Europe. In Croatia the first hybrids were investigated in 1993 (Pospišil and Mustapić, 1995), and introduced into production in 2003.

The nutritive value of rapeseed oils is highly relevant, especially their effect on parameters associated with the development of atherosclerosis. Diseases of the cardiovascular system are the most common cause of death in the industrial countries. The types of fatty acids consumed with the diet affect not only the cholesterol level, but also the susceptibility of low-density lipoproteins to oxidation. Special prominence has been given to the beneficial effects of olive oil in the prevention of cardiovascular disease, primarily due to the high content of monounsaturated acids (MUFA) and native antioxidants (Massaro et al., 2006). Rapeseed oil has also high content of MUFA. The ratio of linoleic acid to linolenic acid affect the risk of atherosclerosis *via* the formation of eicosanoids, thus rapeseed oil can also be effective in preventing atherosclerosis (Eder and Brandsch, 2002; Freese, 2001; Lorgeril et al., 2001). Linoleic and linolenic acids are essential polyunsaturated fatty acids. They are precursors of the other long chain ω-3 and ω-6 polyunsaturated fatty acids (PUFA) which have preventive and therapeutic effects on coronary heart disease and other diseases (Nordoy et al., 2001; Chardigny et al., 2001).

The objective of the present research was to establish the fatty acid composition in oil of rapeseed hybrids in comparison with 00-cultivars, both grown under agroecological conditions of northwestern Croatia in the course of three succesive crop seasons.

Material and methods

Samples

Seven new hybrids ('Artus', 'Baldur', 'Exact', 'Executive', 'Extra', 'RG 9908', 'RG 9909') and eight 00-cultivars ('Aviso', 'Bristol', 'Canary', 'Dexter', 'Ella', 'Kosto', 'Navajo', 'Royal') of rapeseed were grown in the experimental field of the Faculty of Agriculture in Zagreb in the period 2003-2005. Each rapeseed sample was extracted by hexane in laboratory using Soxhlet apparatus, as it is required for quantitative oil content determination (ISO 659:1998). Analyses of oil content and fatty acid composition were carried out in triplicate and the results are elaborated statistically. The mean oil contents (in absolutely dry matter of seed – % of oil in ADM) of rapeseed samples used in experiments were 42.18±3.65% for hybrids and 43,67±4.24% for 00-cultivars.

Chemicals

Standards (fatty acid methyl esters) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade, obtained from Merck KGaA (Darmstadt, Germany) and used without further purification.

Apparatus

Gas chromatography analyses were carried out on ATI Unicam 610 instrument (Cambridge, England) equipped with a split-injector and flame ionization detector (FID).

Fatty acid composition

Methyl-esters of fatty acids (FAME) were prepared using methanolic KOH, according to the standard method (ISO 5509:2000) from the oil obtained after Soxhlet extraction. The fatty acid profile was determined by gas chromatographic separation of their methyl esters (ISO 5508: 1990) on a capillary column (J&W Scientific DB-23, 30 m x 0.25 mm x 0.25 μm). The temperature of the injector and detector was set at 250 °C. The initial oven temperature was 170 °C. This temperature was maintained for 8 min, and then increased at a rate of 2 °C min^{-1} to 190 °C, which was held for 7 min. Helium was used as the carrier gas at a flow rate of 0.87 mL min^{-1} and injection volume was 0.3 μL. The FAME peaks were identified using FAME standards. The fatty acid composition is expressed as weight percentage of total (internal normalization method). Chromatography software (Unicam 4880 chromatography data system) was employed for data collection and processing.

Iodine value

Iodine value was calculated on the basis of fatty acid composition taking into account the percentage of each individual unsaturated fatty acid, as it is described in standard method (AOCS Cd 1c-85).

Statistical analysis

Statistical analysis was performed using the Statistica 7.1. Software. ANOVA was used to determine the effect of seed cultivar and climatic conditions of each investigation year on the fatty acid composition of analyzed oils. The similarity of varieties was tested by Cluster analysis using Ward's method and Euclidian distances (StatSoft, 2005).

Results and discussion

In this work, the results for fatty acid profile and iodine value of all analyzed rapeseed samples from three crop years are presented in Tables 1-6. From that data the Cluster analysis was carried out separately for hybrids and cultivars (Figures 1, 2).

The amounts of most fatty acids were similar in oils from 00 cultivars and in oils from hybrids during three successive crop seasons (Table 1). Oils obtained from 00 cultivars had slightly higher content of oleic and palmitic and lower linoleic and linolenic acids than oils from hybrids. Their average values were inside the data prescribed in Croatian official law regulations (Anon., 1999). There were several samples in which oleic and palmitic acid contents were above and linoleic and linolenic acid contents below the limit values what ought to be incorporated into the earliest revision of present legislation on vegetable oils. Somewhat higher amount of erucic acid had the oils from cultivars but these amounts were far below the established limits. Oils from hybrids had higher amount of polyunsaturated fatty acids (PUFA) as well as iodine value. All samples had low amount of saturated fatty acids (SFA) which is one of the rapeseed oils benefits since SFA have

Table 1. Fatty acid composition (% of total) and iodine value of the oil from all analyzed rapeseed samples from three crop years (2003-2005)*

Fatty acids (% of total)	Hybrids		00-ultivars		Official Regulation of Croatia**
	$\bar{x} \pm \sigma$	Range	$\bar{x} \pm \sigma$	Range	
14:0 Myristic	0.06±0.01	0.05-0.09	0.07±0.02	0.05-0.14	< 0.2
16:0 Palmitic	5.13±0.48	4.11-6.07	5.50±0.51	4.53-6.67	3.3 – 6.0
16:1 Palmitoleic	0.36±0.08	0.18-0.53	0.35±0.05	0.26-0.45	0.1 - 0.6
17:0 Heptadecanic	0.05±0.03	0.00-0.09	0.05±0.02	0.00-0.09	<0.3
17:1 Heptadecenic	0.16±0.02	0.13-0.20	0.16±0.02	0.10-0.19	<0.3
18:0 Stearic	1.48±0.16	1.17-1.95	1.58±0.19	1.30-1.95	1.1 – 2.5
18:1 Oleic	61.88±2.64	57.53-68.93	62.54±3.90	55.92-72.00	52.0 - 66.6
18:2 Linoleic (ω-6)	20.52±1.49	16.01-23.94	19.57±2.51	13.82-24.57	16.1 – 24.8
18:3 Linolenic (ω-3)	8.39±1.50	5.45-11.33	7.92±2.12	4.28-10.58	6.4 - 14.1
20:0 Arachidic	0.52±0.06	0.50-0.59	0.58±0.08	0.45-0.74	0.2 – 0.8
20:1 Gadoleic	1.16±0.11	0.96-1.36	1.29±0.18	1.04-1.87	0.1 – 3.4
22:0 Behenic	0.27±0.07	0.11-0.40	0.31±0.06	0.17-0.44	< 0.5
22:1 Erucic	0.01±0.02	0.00-0.07	0.08±0.15	0.00-0.57	< 2.0
Σ SFA	7.51±0.67	5.98-8.79	8.09±0.75	6.62-9.78	-
Σ MUFA	63.58±2.53	59.48-70.40	64.42±3.89	57.76-73.72	-
Σ PUFA	28.91±2.60	21.46-33.23	27.49±4.37	18.19-34.09	-
Iodine value	112±3.67	102-118	110±6.06	99-118	110 - 126

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids; * mean value±standard deviation; ** Pravilnik o temeljnim zahtjevima za jestiva ulja i masti, margarine i njima slične proizvode, majonezre. umake, preljeve, salate i ostale proizvode na bazi jestivih ulja i masti

Table 2. Saturated fatty acids of the oil from rapeseed hybrids (2003-2005)*

Fatty acid (% of total)	Hybrid						
	Baldur	Artus	Extra	Executive	Exact	RG 9908	RG 9909
14:0	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.01	0.07±0.01	0.07±0.02
16:0[a]	4.98±0.20	5.32±0.13	4.06±0.24	4.49±0.23	5.07±0.16	5.53±0.12	5.71±0.25
17:0[d]	0.06±0.02	0.06±0.02	0.04±0.04	0.04±0.03	0.05±0.04	0.06±0.01	0.05±0.02
18:0[a,f]	1.74±0.12	1.54±0.07	1.35±0.05	1.28±0.06	1.47±0.06	1.51±0.12	1.49±0.09
20:0[b,e]	0.58±0.06	0.55±0.04	0.48±0.06	0.46±0.04	0.51±0.05	0.52±0.02	0.54±0.07
22:0[d]	0.28±0.03	0.30±0.07	0.27±0.05	0.23±0.07	0.26±0.12	0.28±0.06	0.28±0.04
Σ SFA	7.69±0.36	8.03±0.23	6.79±0.33	6.56±0.40	7.42±0.39	7.96±0.29	8.13±0.46

*mean value±standard deviation; SFAs saturated fatty acids; [a] Results are significantly influenced by seed variety ($p \leq 0.001$) [b] Results are significantly influenced by seed variety ($p \leq 0.01$); [c] Results are significantly influenced by seed variety ($p \leq 0.05$); [d] Results are significantly influenced by crop year ($p \leq 0.001$); [e] Results are significantly influenced by crop year ($p \leq 0.01$); [f] Results are significantly influenced by crop year ($p \leq 0.05$)

Table 3. Unsaturated fatty acids and iodine value of the oil from rapeseed hybrids (2003-2005)*

Fatty acid (% of total)	Hybrid						
	Baldur	Artus	Extra	Executive	Exact	RG 9908	RG 9909
16:1[c]	0.36±0.06	0.42±0.07	0.36±0.10	0.28±0.07	0.37±0.08	0.37±0.11	0.37±0.07
17:1[c,f]	0.16±0.03	0.17±0.02	0.17±0.02	0.17±0.02	0.16±0.00	0.16±0.01	0.14±0.01
18:1[b,d]	64.86±2.86	59.31±2.24	61.47±1.98	62.65±1.68	61.19±2.61	61.84±1.67	61.84±2.36
18:2[b,f]	18.04±1.38	20.98±0.88	21.14±0.68	20.42±0.51	22.21±1.25	20.68±0.44	20.17±1.01
18:3[c,d]	7.74±1.77	9.90±1.23	8.85±0.99	8.69±0.80	7.52±1.34	7.86±1.24	8.15±1.77
20:1[d]	1.15±0.09	1.18±0.11	1.20±0.12	1.19±0.10	1.12±0.13	1.12±0.09	1.19±0.11
22:1	0.00±0.00	0.02±0.03	0.01±0.02	0.03±0.03	0.01±0.01	0.01±0.01	0
ΣMUFA	66.53±2.73	61.10±2.05	63.22±1.84	64.33±1.57	62.85±2.53	63.49±1.53	63.54±2.33
ΣPUFA	25.77±3.02	30.87±2.00	30.00±1.65	29.11±1.29	29.73±2.59	28.55±1.65	28.33±2.72
Iodine value[c,d]	109±4	115±3	114±2	113±1	112±4	110.81±2.66	110.74±4.26

*mean value±standard deviation; MUFA monounsaturated fatty acids, PUFAs polyunsaturated fatty acids; [a] Results are significantly influenced by seed variety ($p \leq 0.001$); [b] Results are significantly influenced by seed variety ($p \leq 0.01$); [c] Results are significantly influenced by seed variety ($p \leq 0.05$); [d] Results are significantly influenced by crop year ($p \leq 0.001$) [e] Results are significantly influenced by crop year ($p \leq 0.01$); [f] Results are significantly influenced by crop year ($p \leq 0.05$)

Table 4. Saturated fatty acids of the oil from rapeseed 00-cultivars (2003-2005)*

Fatty acid (% of total)	00-cultivar						
	Bristol	Navajo	Dexter	Ella	Royal	Aviso	Kosto	Canary
14:0	0.08±0.02	0.09±0.03	0.08±0.01	0.07±0.01	0.07±0.01	0.06±0.01	0.06±0.01	0.06±0.01
16:0[b,e]	5.76±0.47	6.12±0.50	5.83±0.34	5.04±0.46	5.51±0.09	5.48±0.27	5.11±0.12	5.12±0.51
17:0[d]	0.05±0.01	0.05±0.01	0.04±0.04	0.05±0.02	0.06±0.02	0.05±0.01	0.06±0.02	0.04±0.01
18:0[c]	1.79±0.12	1.69±0.19	1.80±0.11	1.46±0.14	1.42±0.16	1.44±0.14	1.48±0.04	1.59±0.03
20:0[e]	0.63±0.08	0.57±0.07	0.67±0.08	0.57±0.11	0.51±0.02	0.51±0.07	0.55±0.05	0.59±0.03
22:0[e]	0.37±0.06	0.28±0.04	0.31±0.08	0.26±0.09	0.34±0.05	0.28±0.03	0.30±0.02	0.31±0.05
Σ SFA	8.68±0.75	8.80±0.83	8.74±0.53	7.46±0.67	7.91±0.13	7.82±0.48	7.57±0.13	7.71±0.48

*mean value±standard deviation; SFAs saturated fatty acids; [a] Results are significantly influenced by seed variety ($p \leq 0.001$); [b] Results are significantly influenced by seed variety ($p \leq 0.01$); [c] Results are significantly influenced by seed variety ($p \leq 0.05$); [d] Results are significantly influenced by crop year ($p \leq 0.001$) [e] Results are significantly influenced by crop year ($p \leq 0.01$); [f] Results are significantly influenced by crop year ($p \leq 0.05$)

unfavorable effects on promoting diseases, *e.g.* cardiovascular diseases and atherosclerosis.

With the aim to establish the differences between the samples and influence of crop season the results of three years of investigation are presented as saturated and unsaturated fatty acids, classified in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids separately for hybrids (Tables 2 and 3) and 00-cultivars (Tables 4 and 5).

Table 5. Unsaturated fatty acids of the oil from rapeseed 00-cultivars (2003-2005)*

Fatty acid (% of total)	00-cultivar							
	Bristol	Navajo	Dexter	Ella	Royal	Aviso	Kosto	Canary
16:1 [d]	0.35±0.02	0.37±0.07	0.37±0.05	0.32±0.04	0.38±0.07	0.34±0.03	0.36±0.05	0.30±0.03
17:1 [e]	0.15±0.02	0.14±0.01	0.16±0.02	0.13±0.03	0.17±0.02	0.16±0.02	0.17±0.01	0.17±0.01
18:1 [b,d]	65.53±3.18	62.36±3.98	65.10±3.47	66.48±3.99	58.05±2.55	60.19±2.03	61.45±0.86	61.15±1.57
18:2 [a,f]	17.42±1.90	19.48±1.99	17.63±1.61	16.90±2.22	23.33±1.17	21.09±0.77	20.78±0.43	19.94±0.86
18:3 [c,d]	6.56±2.15	7.11±2.38	6.28±2.18	7.33±2.41	8.95±1.20	9.24±1.70	8.43±1.02	9.49±1.14
20:1 [e]	1.29±0.18	1.41±0.18	1.49±0.28	1.34±0.10	1.21±0.11	1.14±0.05	1.23±0.05	1.23±0.11
22:1 [b]	0.03±0.03	0.33±0.19	0.23±0.15	0.02±0.02	0.00±0.01	0.02±0.02	0.02±0.03	0.00±0.01
ΣMUFA	67.34±3.31	64.61±3.62	67.35±3.31	68.31±3.95	59.81±2.36	61.85±1.94	63.23±0.77	62.86±1.44
ΣPUFA	23.98±4.03	26.59±4.37	23.91±3.78	24.23±4.58	32.28±2.28	30.33±2.41	29.20±0.72	29.43±1.86
Iodine value [c,d]	105±6	108±7	105±6	107±7	115±3	114±4	112±1	113±3

*mean value±standard deviation; MUFA monounsaturated fatty acids, PUFAs polyunsaturated fatty acids; [a] Results are significantly influenced by seed variety ($p \leq 0.001$) [b] Results are significantly influenced by seed variety ($p \leq 0.01$); [c] Results are significantly influenced by seed variety ($p \leq 0.05$); [d] Results are significantly influenced by crop year ($p \leq 0.001$) [e] Results are significantly influenced by crop year ($p \leq 0.01$); [f] Results are significantly influenced by crop year ($p \leq 0.05$)

Table 6. The most represented fatty acids in hybrids during three years*

Year	Fatty acid (% of total)					
	16:0[a]	18:0[a]	18:1[a]	18:2[a]	18:3[a]	22:1[b]
2003	5.01±0.57	1.50±0.22	64.30±2.06	19.77±1.62	7.27±0.98	0.01±0.03
2004	5.27±0.55	1.53±0.13	61.88±1.82	20.53±1.05	7.90±1.24	0.00±0.00
2005	5.10±0.37	1.41±0.12	59.58±1.92	21.28±1.57	9.99±0.70	0.02±0.02

* mean value±standard deviation; [a] Results are significantly influenced by crop year ($p \leq 0.001$) b Results are significantly influenced by crop year ($p \leq 0.01$); [c] Results are significantly influenced by crop year ($p \leq 0.05$)

Table 7. The most represented fatty acids in 00-cultivars during three years*

Year	Fatty acid (% of total)					
	16:0[a]	18:0[c]	18:1[a]	18:2[a]	18:3[a]	22:1[b]
2003	5.73±0.38	1.63±0.17	65.34±3.59	18.47±2.68	6.20±1.40	0.04±0.05
2004	5.62±0.63	1.60±0.25	62.47±3.69	19.51±2.90	7.65±2.21	0.09±0.16
2005	5.15±0.36	1.52±0.13	59.81±2.27	20.73±1.63	9.93±0.53	0.13±0.19

* mean value±standard deviation; [a] Results are significantly influenced by crop year ($p \leq 0.001$) [b] Results are significantly influenced by crop year ($p \leq 0.01$); [c] Results are significantly influenced by crop year ($p \leq 0.05$)

Analysis of variance showed significant influence of cultivars (hybrids) and crop season on fatty acids composition. The amount of oleic acid (and total MUFA) was the highest in hybrid Baldur and cultivar Ella and of essential fatty acids (expressed as PUFA) in hybrid Artus and cultivar Royal. Influence of sample and climatic conditions on fatty acids was statistically significant.

The influence of each crop season on particular fatty acids that could influence the quality and stability of rapeseed oil is shown in Tables 6 and 7. The percentages of oleic acid were the highest and linoleic and linolenic acids were the lowest in the oils from the 2003 crop season which was characterized by higher monthly temperatures and lower rainfall during May and June (Republic of Croatia – Meteorological and Hydrological Service) while lipid biosynthesis is carried out. Environmental factors, such as light, temperature and water stress affect lipid levels and metabolism in the olive fruit (Harwood, 1984) and rapeseed seeds (Gororo et al., 2003).

Results of Cluster analysis according to fatty acid composition are presented in Figure 1 and in Figure 2. Hybrid RG 9908 in crop years 2003 and 2004 (Figure1) as well as cultivars 'Aviso' and 'Royal' in crop years 2004 and 2005 (Figure 2) created homogenous and separated groups. Hybrid RG 9909 in 2003 had similar fatty acid composition as hybrid RG 9908 (2003, 2004). Fatty acid composition of 00 cultivars 'Executive' and 'Extra' was similar through first and second year of investigation. The results

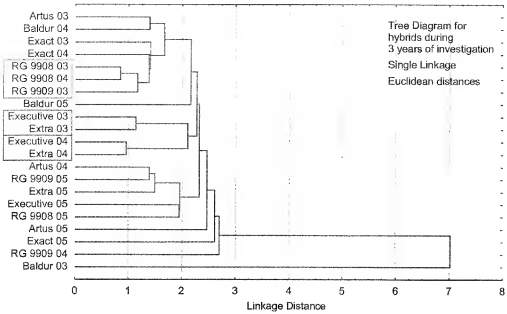

Figure 1.
Cluster analysis of rapeseed hybrids according fatty acid composition

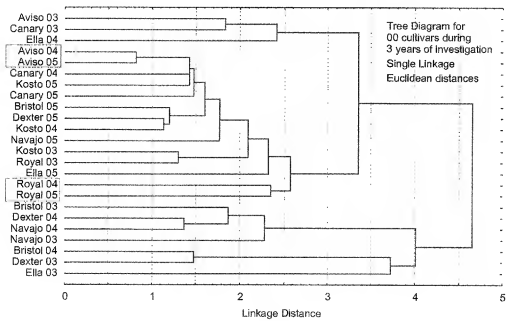

Figure 2.
Cluster analysis of rapeseed 00-cultivars according fatty acid composition

of the other samples showed wide distribution of data thus fatty acid composition by itself cannot be helpful in characterization of these oils therefore it has to be completed with other analyses.

Conclusion

In view of the performed research on seven new hybrids and eight 00-cultivars of rapeseed in the period 2003 -2005, grown in the experimental field of the Faculty of Agriculture in Zagreb, and analyzed results it could be concluded that the oil content in the seed from 00-cultivars was higher than in the seed from hybrids during three years of investigation.

In both investigated groups there were no significant differences in composition of those fatty acids which could influence the quality and stability of rapeseed oil. Neither hybrids nor 00-cultivars contained erucic acid or it was present far bellow the limit allowed in law regulations (2%). The average values of individual fatty acids in oils obtained from hybrids as well as from 00-cultivars are inside the prescribed limits, but there were several samples

in which oleic and palmitic acid contents were above and linoleic and linolenic acid contents (as well as the iodine values) below the limit values. This ought to be taken into consideration at the earliest revision of official regulations on vegetable oils.

Investigated rapeseed hybrids as well as 00-cultivars contained much the same amount of total monounsaturated (MUFA), total polyunsaturated (PUFA) and total saturated (SFA) fatty acids. The high content of oleic acid and low content of saturated fatty acids are very important characteristics of rapeseed oil, and, combined with the presence of linoleic and linolenic acid, responsible for its unique nutritive value and efficiency in preventing of cardiovascular diseases.

Weather conditions in each year greatly influenced fatty acid composition of all investigated samples what was confirmed by variance analysis.

The determination of fatty acid composition by itself cannot be helpful in characterization of rapeseed oil; therefore it has to be combined with other analyses.

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acs72_30

Rapid Discrimination of Fatty Acid Composition in Fats and Oils by Electrospray Ionization Mass Spectrometry

Shoji KURATA,*† Kazutaka YAMAGUCHI,* and Masatoshi NAGAI**

**Criminal Investigation Laboratory, Metropolitan Police Department, 2-1-1, Kasumigaseki, Chiyoda-ku, Tokyo 100-8929, Japan*

***Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Nakanachi, Koganei, Tokyo 184-8588, Japan*

Fatty acids in 42 types of saponified vegetable and animal oils were analyzed by electrospray ionization mass spectrometry (ESI-MS) for the development of their rapid discrimination. The compositions were compared with those analyzed by gas chromatography-mass spectrometry (GC-MS), a more conventional method used in the discrimination of fats and oils. Fatty acids extracted with 2-propanol were detected as deprotonated molecular ions ($[M-H]^-$) in the ESI-MS spectra of the negative-ion mode. The composition obtained by ESI-MS corresponded to the data of the total ion chromatograms by GC-MS. The ESI-MS analysis discriminated the fats and oils within only one minute after starting the measurement. The detection limit for the analysis was approximately 10^{-10} g as a sample amount analyzed for one minute. This result showed that the ESI-MS analysis discriminated the fats and oils much more rapidly and sensitively than the GC-MS analysis, which requires several tens of minutes and approximately 10^{-9} g. Accordingly, the ESI-MS analysis was found to be suitable for a screening procedure for the discrimination of fats and oils.

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Introduction

A variety of fats and oils are widely available for consumption in the form of cooking oils, health products and food ingredients, as well as for the manufacture of cosmetics, soaps and surfactants. These fats and oils primarily contain triacylglycerols (also known as triglycerides), which are esters derived from the reaction of the carboxylic functional groups of fatty acid with each of the alcohol functional groups of glycerol. Fatty acid compositions vary among the various types of fats and oils. Fatty acid compositions play an important role in forensic science, sometimes serving as evidence in criminal investigations into the causes of fires resulting from arson or spontaneous ignition.[1-3] Discrimination between control oil samples, taken from a suspect or victim, and oil samples collected at crime scenes is required, as is the identification of their types in certain cases. When a control fat or oil is unknown, the fatty acid composition (including the degree of unsaturation) and the varying chain lengths are useful for establishing the distinction between liquid oils and solid fats; the classification into drying, semi-drying, and nondrying oils; and the discrimination of types of fats and oils. Fatty acid composition is frequently analyzed by gas chromatography (GC)[4-10] and gas chromatography-mass spectrometry (GC-MS)[4,5,11-14] of saponifiables[4,7,8,9,12-13] for their classification, or for the discrimination of fats and oils. When fats and oils are saponified, saponifiables are obtained in the form of fatty acid salts, such as potassium salts in an aqueous layer. They are acidified, and then extracted as free fatty acids with organic solvent. This sample preparation is effective in removing impurities from polluted fats and oils. The analysis of the GC or GC-MS for fatty acid requires esterification or transesterification, which is a laborious and time-consuming exercise in the examination procedure. The measurement times of GC and GC-MS analyses require at least a few tens of minutes for the elution and separation of the saponifiables of the fats and oils. Some fats and oils, particularly castor oil and fish oils, take longer to analyze than others because they contain more types of fatty acids. Recently, electrospray ionization mass spectrometry (ESI-MS) has been applied to the analysis of the fatty acid in both the positive[15-18] and negative[18,19] ion modes. The saponifiables of the fats and oils (fatty acids) transform relatively easily into negative ions due to the compounds' carboxyl group. The ESI-MS analysis of the fatty acids resulting from the saponification is therefore expected to be suitable for a screening procedure for the rapid discrimination of fats and oils. In this paper, we describe a new, faster, easier method that enables the analysis of a number of fat and oil samples by ESI-MS over a short period of time. The analysis of the saponifiables of 42 types of vegetable and animal oils for the development of a screening procedure for the discrimination of fats and oils are reported in this work. The sample preparation was reduced to only two steps: saponification and extraction without esterification or transesterification. The GC-MS analysis of the saponifiables was also carried out to compare the analytical results.

Experimental

Materials

Fat and oil manufacturers in Japan supply 27 types of

† To whom correspondence should be addressed.
E-mail: shkurata@jcom.home.ne.jp

Table 1 Fatty acid composition (%) of fats and oils by electrospray ionization mass spectrometry

Vegetable oils No. 1

Fatty acid	[M–H]⁻ (*m/z*)	Coconut oil	Palm kernel oil	Palm oil	Camellia oil	Macadamia nut oil	Bran oil	Rapeseed oil	Olive oil	Tea seed oil	Peanut oil
C8:0	143	5.1	3.1	—	—	—	—	—	—	—	—
C10:0	171	5.1	3.9	—	—	—	—	—	—	—	—
C12:0	199	55.4	58.8	0.8	—	—	—	—	—	—	—
C14:0	227	19.6	15.5	1.0	trace	0.8	0.5	—	—	0.4	—
C16:0	255	6.5	5.4	15.2	11.5	7.2	11.6	5.0	11.3	14.7	8.4
C16:1	253	—	—	—	0.9	27.7	0.5	0.4	1.0	0.5	—
C18:0	283	2.2	1.6	1.0	1.1	0.9	trace	0.8	2.2	1.0	0.8
C18:1	281	6.3	10.1	63.4	69.9	58.6	45.7	58.9	78.2	63.5	48.0
C18:2	279	1.1	1.6	18.6	10.2	1.5	39.2	21.8	6.2	11.7	37.9
C18:3	277	—	—	—	0.9	—	1.8	8.5	—	—	0.6
C20:0	311	—	—	—	—	0.8	—	0.5	—	—	0.6
C20:1	309	—	—	—	0.9	2.3	0.6	1.5	—	1.2	0.6
C22:1	337	—	—	—	—	—	—	0.5	—	—	—
Other		0.7	trace	trace	4.6	0.2	0.1	2.1	1.1	7.0	3.1
Total		100	100	100	100	100	100	100	100	100.0	100

Trace: < 0.1%, —: not detected.

Vegetable oils No. 2

Fatty acid	[M–H]⁻ (*m/z*)	Cocoa butter	Tall oil	Safflower oil	Cotton seed oil	Soybean oil	Evening primrose oil	Corn oil	Sesame oil	Sunflower oil	Grape seed oil
C12:0	199	—	0.3	—	—	—	—	—	—	—	—
C14:0	227	—	0.4	—	1.2	—	—	—	—	—	—
C16:0	255	10.3	3.0	6.8	14.1	7.3	4.8	9.2	6.0	4.5	6.2
C16:1	253	trace	0.5	—	0.8	—	—	0.3	0.5	—	—
C18:0	283	4.0	trace	0.8	0.4	1.0	0.8	0.9	2.0	1.7	2.2
C18:1	281	48.9	27.8	9.2	17.5	29.9	6.2	32.5	44.1	19.2	17.0
C18:2	279	4.6	20.2	80.5	62.9	53.3	66.3	53.6	46.5	69.1	67.3
C18:3	277	1.3	2.8	1.0	0.6	6.5	10.9	2.1	0.5	0.9	0.9
C20:0	311	—	—	—	—	—	0.9	0.3	0.3	trace	1.3
C20:1	309	—	0.3	—	—	—	—	0.3	—	trace	trace
C20:3	305	—	2.8[a]	—	—	—	—	—	—	—	—
C20:5	301	—	30.6[a]	—	—	—	—	—	—	—	—
C20:6	299	—	6.3[a]	—	—	—	—	—	—	—	—
C24:1	365	2.5	—	—	—	—	—	—	—	—	—
Other		28.4	5.0	1.7	2.5	2.0	10.1	0.8	0.1	14.6	5.1
Total		100	100	100	100	100	100	100	100	100	100

a. Resin acids.

Vegetable oils No. 3

Fatty acid	[M–H]⁻ (*m/z*)	Cayenne oil	Perilla oil	Egoma seed oil	Linseed oil	Jojoba oil	Tung oil	Castor oil	Turkey red oil
[Cp–137]⁻	168	14.8	—	—	—	—	—	—	—
[DC–137]⁻	170	19.4	—	—	—	—	—	—	—
C10:0	171	2.8	—	—	—	—	trace	—	—
C12:0	199	1.5	—	—	—	—	—	—	—
C14:0	227	2.7	—	—	—	—	—	—	—
C14:1	225	trace	trace	—	—	—	1.3	—	—
C15:0	241	2.5	—	—	—	—	—	—	—
C15:1	239	0.8	—	—	—	—	—	—	—
C16:0	255	6.1	4.7	7.5	6.1	1.3	5.0	0.5	1.0
C16:1	253	2.5	—	—	—	0.7	—	—	—
C17:0	269	0.9	—	—	—	—	—	—	—
C18:0	283	0.6	1.3	1.1	1.8	—	2.9	0.4	0.5
C18:1	281	8.3	22.6	14.7	25.5	17.9	16.5	2.9	2.9
C18:2	279	17.1	16.8	12.3	14.6	trace	28.1	4.9	1.7
C18:3	277	6.5	47.1	59.4	46.7	—	30.9[b]	0.4	—
C18:4	275	trace	1.2	0.8	1.5	—	0.8	—	—
Cp	306	2.7	—	—	—	—	—	—	—
DC	306	2.8	—	—	—	—	—	—	—
C20:0	311	trace	—	—	—	—	6.2	—	—
C20:1	309	—	—	—	—	50.4	2.6	—	—
C22:1	337	—	—	—	—	11.3	—	—	—
C24:1	365	—	—	—	—	1.3	—	—	—
[RA–114]⁻	183	—	—	—	—	—	—	14.8	6.8
[RA]⁻	297	—	—	—	—	—	—	75.2	57.5
[RA–118]⁻	415	—	—	—	—	—	—	—	23.7
Other		8.0	6.3	4.0	3.8	17.1	5.7	0.9	5.9
Total		100	100	100	100	100	100	100	100

b. Eleostearates. Cp, capsaicin; DC, dihydrocapsaicin; RA, ricinoleic acid.

Animal oils No. 1

Fatty acid	[M–H]⁻ (*m/z*)	Turtle oil	Whale oil	Shark liver oil	Mackerel oil	Sardine oil	Cod liver oil	Saury oil	Cuttlefish oil	Beef tallow	Lard
C12:0	199	18.3	1.8	—	—	—	—	—	—	—	—
C12:1	197	—	0.7	—	—	0.3	—	—	—	—	—
C14:0	227	8.5	7.0	1.7	6.9	17.1	7.3	9.3	4.3	4.0	2.4
C14:1	225	0.9	4.6	—	—	0.7	0.3	0.5	—	2.5	1.0
C15:0	241	—	0.7	0.3	0.7	1.0	0.6	1.1	0.6	0.7	—
C16:0	255	9.6	7.4	8.7	9.1	18.1	11.7	8.3	9.9	10.9	10.5
C16:1	253	8.5	20.2	7.1	7.1	11.4	10.8	7.1	3.9	6.5	5.4
C17:0	269	—	—	—	—	1.0	—	trace	—	1.0	—
C17:1	267	—	2.8	1.7	0.9	0.5	0.6	1.4	3.7	1.5	0.5
C17:2	265	—	—	—	—	—	—	—	0.6	1.2	—
C18:0	283	1.4	0.9	3.3	2.6	1.1	1.7	1.8	6.2	2.5	2.0
C18:1	281	33.5	25.7	22.4	18.0	10.1	21.0	7.7	11.2	59.7	58.0
C18:2	279	13.9	1.5	1.7	1.9	0.8	2.5	2.5	1.2	6.0	13.7
C18:3	277	1.3	—	0.9	1.9	0.3	0.8	2.1	—	—	0.7
C18:4	275	—	—	0.9	5.7	—	1.7	6.1	—	—	—
C20:0	311	—	—	—	—	0.6	1.9	trace	—	—	—
C20:1	309	—	12.1	8.2	7.8	15.2	10.0	16.3	14.0	0.5	0.7
C20:3	305	0.9	—	—	—	—	—	trace	—	—	—
C20:4	303	0.9	—	7.2	1.8	—	1.3	2.0	2.3	—	—
C20:5	301	—	2.5	8.5	10.1	0.3	9.5	7.6	9.1	—	—
C22:1	337	—	4.8	3.2	9.7	16.1	9.2	16.1	7.5	—	—
C22:5	329	0.4	0.7	4.7	1.6	trace	0.6	1.7	trace	—	—
C22:6	327	—	2.2	17.4	12.1	—	4.8	6.6	23.7	—	—
C24:1	365	—	0.8	0.9	0.9	1.0	1.1	1.2	1.5	—	0.7
Other		1.9	3.6	1.2	1.2	4.4	2.6	0.6	0.3	3.0	4.4
Total		100	100	100	100	100	100	100	100.0	100	100

Animal oils No. 2

Fatty acid	[M–H]⁻ (*m/z*)	Mutton tallow	Chicken fat	Waterfowl liver oil	Butter
C6:0	115	—	—	—	15.3
C8:0	143	—	—	—	6.3
C10:0	171	trace	—	—	8.1
C10:1	169	—	—	—	1.9
C12:0	199	trace	—	—	6.9
C14:0	227	5.3	1.6	3.3	8.1
C14:1	225	0.7	—	—	3.1
C15:0	241	2.1	—	0.4	2.7
C16:0	255	15.4	24.3	10.1	7.8
C16:1	253	3.0	7.3	11.4	trace
C17:0	269	2.2	—	0.4	1.7
C17:1	267	0.7	—	1.1	—
C18:0	283	10.9	3.9	3.0	2.5
C18:1	281	44.9	43.9	28.0	7.4
C18:2	279	5.7	15.8	1.3	—
C18:3	277	1.9	0.8	0.4	—
C18:4	275	—	—	1.1	—
C20:0	311	1.1	—	0.9	1.4
C20:1	309	trace	0.4	8.4	—
C20:4	303	—	—	1.5	—
C20:5	301	—	—	9.0	—
C22:1	337	—	—	7.3	—
C22:5	329	—	—	1.5	—
C22:6	327	—	—	8.0	—
C24:1	365	—	—	1.4	—
Other		6.1	2.0	1.5	26.8
Total		100	100	100	100

vegetable oils and nine types of animal oils tested in this experiment. Cayenne oil, mutton tallow, cuttlefish oil, mackerel oil, shark liver oil, and saury oil extracted with boiling, distilled water were used in the analysis. The potassium hydroxide (85%), sodium sulfate (99%), sulfuric acid (97%), ethanol (99.5%), and diethyl ether (99.5%) used were of analytical grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Distilled water and 2-propanol were of HPLC grade (Wako Pure Chemical Industries, Ltd.). Diazomethane was prepared from 5 g of nitrosomethylurea and 15 mL of 50% potassium hydroxide in 50 mL of ether.[31]

Saponification of fats and oils for sample preparation

Samples of fats and oils (approximately 0.2 g) were saponified with 0.5 M potassium hydroxide-ethanol solution (3 mL).[4,5,11-13,22,23] The solution was heated for 1 h at 70°C. After saponification, the solution was divided into two samples for GC-MS and ESI-MS analyses. To a solution containing the saponified products for the GC-MS analysis were added 10 mL of water. The unsaponifiables were extracted from the aqueous solution twice with 6 mL of diethyl ether. The aqueous solution was acidified with sulfuric acid (1 M). The saponifiables were extracted from the aqueous solution twice with 6 mL of diethyl ether. Then, 12 mL of a diethyl ether solution was washed with 10 mL of distilled water and dried over anhydrous sodium sulfate. The resulting fatty acids were methylated with an ethereal solution of diazomethane[32,33] as a sample for injection into GC-MS. The other saponified solution for ESI-MS analysis was evaporated to dryness in a water bath at 70°C. The saponifiables were extracted with 2 mL of 2-propanol. The solution was diluted with 2-propanol as a sample for injection into ESI-MS.

ESI-MS analysis

Infusion ESI-MS analysis was performed using a Waters Alliance system coupled with a Micromass ZMD mass spectrometer. Samples in a 250 µl syringe (Hamilton Co., Reno, Nevada) were delivered at 20 µl/min by a Harvard Apparatus 11 syringe pump to an ESI probe connected to a 100-µm-i.d. fused silica capillary (phenylmethyl-deactived GC guard column, Restek Corp., Bellefonte, Pennsylvania). The mass spectra were determined in the negative-ion mode with a mass-to-charge ratio (*m/z*) range of 100 – 500, which detected fatty acids with six (C6:0) or more carbon numbers, as shown in Table 1. The ESI conditions were as follows: nitrogen, 400 L/h; capillary voltage, 3.0 kV; source block temperature, 140°C; desolvation temperature, 400°C; LM resolution, 13.9; HM

Table 2 Fatty acid composition (%) of fats and oils by gas chromatography–mass spectrometry

Vegetable oils No. 1

Fatty acid	Retention time/min	Coconut oil	Palm kernel oil	Palm oil	Camellia oil	Macadamia nut oil	Bran oil	Rapeseed oil	Olive oil	Tea seed oil	Peanut oil
C10:0	1.8	10.3	11.7	0.5	trace	—	0.5	—	—	0.3	0.2
C12:0	2.2	59.1	69.3	1.1	—	0.1	—	—	—	—	0.2
C14:0	2.8	17.9	9.7	2.2	trace	0.8	0.4	trace	—	0.1	0.1
C15:0	3.3	—	—	—	—	—	—	—	—	0.1	—
C16:0	4.1	7.2	2.3	43.1	9.6	9.4	18.8	4.9	11.1	21.1	16.7
C16:1*n*–7	4.5	—	—	0.2	trace	22.2	0.2	0.2	0.7	0.1	—
C17:0	5.1	—	—	—	trace	—	—	—	0.1	0.1	—
C18:0	6.5	2.0	0.3	3.0	2.3	3.5	1.8	1.8	3.2	3.2	3.3
C18:1*n*–9	7.0	3.1	2.2	40.3	85.0	51.0	42.6	56.8	76.0	58.9	43.4
C18:1*n*–7	7.1	—	—	trace	trace	4.5	—	4.5	—	—	—
C18:2*n*–6	8.0	0.3	0.4	9.0	2.5	1.7	33.6	20.5	7.4	12.5	34.4
C18:3*n*–6	8.9	—	—	0.1	—	—	—	trace	0.3	—	—
C18:3*n*–3	9.4	—	—	—	—	—	0.8	9.0	—	—	—
C20:0	11.1	—	—	—	—	2.7	0.4	0.4	0.4	—	0.4
C20:1*n*–9	11.9	—	—	—	trace	2.4	0.3	1.3	0.3	0.6	0.2
Other		0.1	4.1	0.5	0.6	1.7	0.6	0.6	0.5	3.0	1.1
Total		100	100	100	100	100	100	100	100	100	100

Trace: < 0.1%. —: not detected.

Vegetable oils No. 2

Fatty acid	Retention time/min	Cocoa butter	Tall oil	Safflower oil	Cotton seed oil	Soybean oil	Evening primrose oil	Corn oil	Sesame oil	Sunflower oil	Grape seed oil
C10:0	1.8	—	—	—	trace	—	—	—	—	0.1	—
C12:0	2.2	0.4	—	trace	trace	—	—	—	—	—	—
C14:0	2.8	0.1	0.9	0.1	0.8	trace	—	0.1	—	0.1	—
C16:0	4.1	28.9	13.1	6.6	24.1	11.9	8.0	12.3	9.7	7.3	7.4
C16:1*n*–7	4.5	0.3	1.2	trace	0.5	trace	—	—	0.1	—	trace
C17:0	5.1	—	—	trace	trace	trace	—	—	—	0.1	—
C18:0	6.5	25.7	1.6	2.5	2.2	4.5	2.1	2.1	6.1	4.4	4.3
C18:1*n*–9	7.0	39.8	47.8	13.1	18.6	24.8	9.7	32.6	40.6	22.3	18.6
C18:1*n*–7	7.1	—	trace	0.2	trace	1.4	0.8	—	—	0.2	1.0
C18:2*n*–6	8.0	2.9	29.0	76.0	52.7	45.6	71.6	47.5	41.8	63.6	67.5
C18:3*n*–6	8.9	—	—	0.1	trace	0.8	7.3	trace	0.2	—	trace
C18:3*n*–3	9.4	—	—	0.3	0.2	9.3	0.1	1.7	0.4	0.7	0.3
C20:0	11.1	trace	—	0.3	trace	0.3	0.2	trace	0.6	0.2	0.1
C20:1*n*–9	11.9	—	0.7	—	—	0.2	—	trace	—	0.1	0.1
Other		1.9	5.7	0.8	0.9	1.2	0.2	3.7	0.5	0.9	0.7
Total		100	100	100	100	100	100	100	100	100	100

Vegetable oils No. 3

Fatty acid	Retention time/min	Cayenne oil	Perilla oil	Egoma seed oil	Linseed oil	Jojoba oil	Tung oil	Castor oil	Turkey red oil
C10:0	1.8	0.6	—	—	—	—	—	—	—
C12:0	2.2	1.3	—	0.1	—	—	0.1	—	—
C14:0	2.8	4.2	0.1	trace	—	—	trace	—	—
C15:0	3.3	4.5	trace	—	—	—	—	—	—
C16:0	4.1	20.4	7.1	6.6	6.9	1.9	3.3	1.4	5.8
C16:1*n*–7	4.5	1.8	0.1	trace	0.4	0.2	—	—	—
C17:0	5.1	0.6	trace	—	—	—	—	—	—
C18:0	6.5	2.9	4.5	1.5	4.2	0.1	2.4	1.5	1.9
C18:1*n*–9	7.0	12.5	20.4	14.4	24.3	10.4	5.2	4.1	11.1
C18:1*n*–7	7.1	2.1	trace	—	—	0.6	trace	trace	trace
C18:2*n*–6	8.0	27.7	16.6	15.1	17.9	0.6	8.0	4.3	3.6
C18:3*n*–6	8.9	—	trace	0.2	0.6	—	—	—	—
C18:3*n*–3	9.4	10.6	50.4	60.1	44.1	trace	—	0.3	—
C20:0	11.1	0.8	trace	0.1	—	—	—	—	—
C20:1*n*–9	11.9	—	0.2	0.4	—	66.6	0.4	—	—
C18:3*n*–5	19.4	—	—	—	—	—	64.1	—	—
C22:0	20.2	0.9	—	—	—	—	—	—	—
C22:1*n*–9	21.2	—	—	0.3	—	9.7	—	—	—
C18:3*n*–5	21.7	—	—	—	—	—	13.8	—	—
RA	37.7	—	—	—	—	—	—	87.7	65.5
Other		9.1	0.6	1.2	1.6	9.9	2.7	0.7	12.1
Total		100	100	100	100	100	100	100	100

RA: ricinoleic acid.

Animal oils No. 1

Fatty acid	Retention time/min	Turtle oil	Whale oil	Shark liver oil	Mackerel oil	Sardine oil	Cod liver oil	Saury oil	Cuttlefish oil	Beef tallow	Lard
C10:0	1.8	—	2.0	—	—	0.6	—	—	—	0.6	2.0
C12:0	2.2	23.2	4.8	—	0.1	0.2	0.4	trace	—	0.1	0.7
C14:0	2.8	12.5	17.0	2.7	9.9	18.1	5.1	7.1	4.7	3.8	6.1
C14:1*n*–5	3.1	0.7	3.9	0.1	0.1	trace	0.2	0.3	0.2	1.1	1.3
C15:0	3.3	0.2	0.8	0.3	0.4	0.5	0.3	0.6	0.5	0.4	0.4
C16:0	4.1	18.0	14.1	25.7	18.8	23.5	11.7	11.1	17.8	26.4	26.3
C16:1*n*–7	4.5	6.9	16.6	6.1	5.2	13.2	7.9	3.5	2.5	3.1	4.4
C17:0	5.1	—	1.2	1.0	0.4	0.9	1.8	0.6	3.0	1.2	0.5
C18:0	6.5	2.8	1.1	2.7	2.6	1.8	2.0	1.6	4.7	16.9	8.6
C18:1*n*–11	6.9	—	1.1	0.5	—	—	1.0	0.5	—	3.0	trace
C18:1*n*–9	7.0	22.3	16.8	16.5	16.1	10.7	14.1	4.6	9.0	36.1	27.3
C18:1*n*–7	7.1	2.7	1.6	6.4	3.3	2.6	5.0	1.2	2.4	1.8	1.7
C18:2*n*–6	8.0	8.5	0.6	1.3	1.4	1.5	2.1	1.6	0.8	2.2	5.8
C18:3*n*–3	9.4	0.4	—	0.5	1.4	0.1	0.8	1.6	0.3	—	—
C20:1*n*–11	11.7	—	1.4	3.8	0.3	9.8	6.3	12.6	7.0	—	—
C20:1*n*–9	11.9	—	1.4	2.7	7.1	3.0	3.7	3.6	5.0	—	—
C20:5*n*–3	19.2	—	—	6.0	6.6	—	10.0	7.2	7.4	—	—
C22:1*n*–11	20.9	—	0.5	1.2	7.6	9.6	8.4	18.8	4.5	—	—
C22:6*n*–3	36.6	—	—	13.0	9.9	—	6.7	8.3	22.8	—	—
Other		1.8	15.1	9.5	8.8	4.1	12.5	15.2	7.4	3.3	4.9
Total		100	100	100	100	100	100	100	100	100	100

Animal oils No. 2

Fatty acid	Retention time/min	Mutton tallow	Chicken fat	Waterfowl liver oil	Butter
C9:0	1.7	—	—	—	3.2
C10:0	1.8	0.1	—	0.2	13.7
C11:0	1.9	—	—	trace	2.4
C12:0	2.2	0.2	trace	0.3	10.2
C14:0	2.8	3.2	1.3	2.6	17.5
C14:1*n*–5	3.1	0.3	0.2	0.1	0.7
C15:0	3.3	0.7	0.1	0.4	1.9
C16:0	4.1	20.1	27.0	11.1	27.3
C16:1*n*–7	4.5	1.0	5.7	8.3	—
C17:0	5.1	1.6	0.1	1.0	0.5
C18:0	6.5	25.8	5.3	3.3	8.1
C18:1*n*–11	6.9	5.0	trace	1.4	—
C18:1*n*–9	7.0	33.0	42.0	18.1	4.9
C18:1*n*–7	7.1	trace	1.8	8.6	—
C18:2*n*–6	8.0	1.9	15.2	0.8	—
C18:3*n*–3	9.4	1.5	0.4	0.3	—
C20:1*n*–11	11.7	—	—	4.5	—
C20:1*n*–9	11.9	—	—	3.3	—
C20:5*n*–3	19.2	—	—	8.6	—
C22:1*n*–11	20.9	—	—	2.9	—
C22:6*n*–3	36.6	—	—	10.4	—
Other		5.6	0.9	13.8	9.6
Total		100	100	100	100

resolution, 13.9; ion energy, 0.5 V; and multiplier, 650 V. To optimize the spectra, the cone voltage spanned 10, 20, 40, 60, 80, and 120 V. The measurement time for each sample was set at 1 min. Although all of the spectra in the cone voltage range of 10 – 120 V were similar, the optimum cone voltage was 40 V. The cone voltage for the ESI-MS analysis of other fats and oils was set at 40 V. The detection limit of the oil for identification was 1×10^{-8} g/mL, which corresponded to 2×10^{-10} g as the sample amount analyzed for a 1-min measurement. The fatty acid compositions were calculated on the basis of the ratios of the peak height intensity in the ESI-MS spectra in the analysis of the isotope effect as follows. The major peaks, such as octadecenoic acid (C18:1), influenced the percentage calculation of the minor peaks, such as stearic acid (C18:0), because the infusion ESI-MS could not separate the compounds at the same mass number, such as the peak of C18:0 and the isotopic peak of C18:1 at *m/z* 283. On the other hand, the isotopic peaks were observed in the *m/z* region from "M – 1" to "M + 1" in the ESI-MS spectra of the negative-ion mode of fatty acids with the molecular weight "M". The peak at *m/z* "M – 1" was a base peak. In the case of measuring the ESI-MS spectra in the negative mode of fatty acids, the influence of the isotope effect must be considered between two compounds with a mass difference of 2 mass units, such as C18:0 and C18:1. The ratio percentages of the isotopic peaks at *m/z* "M + 1" to the base peaks at *m/z* "M – 1" (molecular weight "M") of fatty acids (C14:1, C16:1, C18:1, C20:1, and C22:1 in Table 1) were approximately 1.6, 2.0, 2.4, 2.9, and 3.3%, respectively, using an isotope modelling tool in Micromass MassLynx 3.3. These isotopic peaks overlapped with the base peaks of C14:0, C16:0, C18:0, C20:0, C22:0 (molecular weight "M + 2") in Table 1 at *m/z* "M + 1", respectively. All of the fatty acids C18:0, C18:1, C18:2, and C18:3 showed almost the same ratio percentages (approximately 2.4%) of the isotopic peaks at *m/z* "M + 1" to base peaks at *m/z* "M – 1" of the fatty acids with the same carbon number in Table 1. The influence of the isotopic effect between the compounds with a mass difference of 4 or more mass units were not observed in the ESI-MS spectra obtained with the isotope modeling tool.

GC-MS analysis

A GC-MS analysis was performed using a Shimadzu GCMS-QP2010. A fused-silica capillary column (DB23 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) (J & W Scientific, Folsom, California)) was used to separate fatty acid methyl esters. The flow rate of the helium carrier gas was 1 mL/min. Samples were injected using a split mode, and the split ratio was 1:50. The oven temperature was held at 180°C for 40 min. The temperatures of the transfer line and the injector were 230°C and 200°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV and in the scan range of 35 – 600 *m/z*. The compounds measured by GC-MS in this study were the methyl esters of the fatty acids with nine (C9:0) or more carbon numbers, as shown in Table 2, owing to the

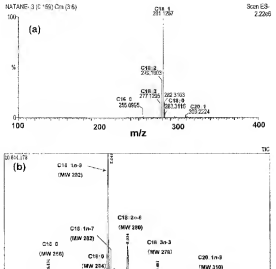

Fig. 1 Mass spectrum by ESI-MS analysis (a) and total ion chromatogram by GC-MS analysis (b) of rapeseed oil. In the mass spectrum; C16:0, palmitic acid; C18:0, stearic acid; C18:1, octadecenoic acid; C18:2, octadecadienoic acid; C18:3, octadecatrienoic acid; C20:1, icosenoic acid. In the total ion chromatogram, C16:0, methyl palmitate; C18:0, methyl stearate; C18:1*n*–9, methyl oleate, C18:1*n*–7, methyl vaccenate; C18:2*n*–6, methyl linoleate; C18:3*n*–3, methyl linolenate; C20:1*n*–9, methyl icosenoate.

solvent delay. The detection limit for discrimination of the fats and oils was 2×10^{-8} g. The fatty acid compositions were calculated on the basis of the peak-area intensity ratios in total ion chromatograms of GC-MS.

Results and Discussion

Comparison between fatty acid composition by ESI-MS and that by GC-MS.

The mass spectrum by the ESI-MS analysis of fatty acids in a saponified rapeseed oil appears in Fig. 1(a). The ESI-MS spectra of fatty acids in the saponified fats and oils were detected as deprotonated molecular ions ($[M-H]^-$). The isomers of unsaturated fatty acids were detected as a summed peak by ESI-MS analysis. The major unsaturated fatty acids of the oil detected in the mass spectrum were octadecenoic acid (C18:1, m/z = 281) composed of oleic acid and a small amount of vaccenoic acid, octadecadienoic acid (C18:2, m/z = 279) corresponding to linoleic acid, and octadecatrienoic acid (C18:3, m/z = 277) corresponding to linolenic acid with minor amounts of icosenoic acid (C20:1, m/z = 309). The saturated fatty acids were palmitic acid (C16:0, m/z = 255) and minor amounts of stearic acid (C18:0, m/z = 283). For a comparison, a total ion chromatogram of the fatty acid methyl esters from the saponified rapeseed oil by the GC-MS analysis is shown in Fig. 1(b). The major unsaturated fatty acid methyl esters detected in the total ion chromatogram were methyl oleate (C18:1*n*–9), methyl linoleate (C18:2*n*–6), and methyl linolenate (C18:3*n*–3) with minor amounts of methyl vaccenate (C18:1*n*–7) and

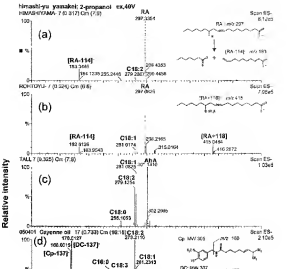

Fig. 2 Mass spectra by ESI-MS analysis of castor oil (a), turkey red oil (b), tall oil (c), cayenne oil (d) and tung oil (e). RA, ricinoleic acid; $[RA-114]^-$, the decomposition product negative ion of ricinoleic acid; $[RA+118]^-$, a negative ion of a potassium sulfated ricinoleic acid; AbA, abietic acid; Cp, capsaicin; DC, dihydrocapsaicin; $[Cp-137]^-$, fragment ion of capsaicin; $[DC-137]^-$, fragment ion of dihydrocapsaicin.

methyl icosenoate (C20:1*n*–9). The saturated fatty acid methyl esters included methyl palmitate (C16:0) and minor amounts of methyl stearate (C18:0). The fatty acid composition of the saponified rapeseed oil by ESI-MS analysis was very similar to that by the GC-MS analysis. The fatty acid compositions of the 42 types of saponified vegetable and animal oils by ESI-MS analysis and by GC-MS analysis are shown in Tables 1 and 2, respectively. With the exception of castor oil, turkey red oil, tall oil, and cayenne oil, the analytical results of the 38 types of fats and oils showed that the fatty acid compositions detected by ESI-MS analysis corresponded to those by GC-MS analysis.

Castor oil. In the ESI-MS spectrum of castor oil (Fig. 2(a)) the deprotonated molecular negative ions of ricinoleic acid, octadecadienoic acid, octadecenoic acid, palmitic acid, stearic acid and octadecatrienoic acid were all identified. They were also detected by the GC-MS analysis (Fig. 3(a)) and were observed with a peak (14.8%) at m/z of 183. The deprotonated molecular negative ions of ricinoleic acid in Fig. 2(a) are characteristic of castor oil. The peak at m/z 183 was assigned to a decomposition product with a negative ion $[M-114]^-$ of ricinoleic acid (Fig. 2(a)). F.-F. Hsu *et al.* report that a basic peak in a tandem mass spectrum of the dilithiated adducts of ricinoleic acid by the ESI-MS-MS analysis in the positive mode is a decomposition product positive ion, $[M-114+2Li]^+$, of ricinoleic acid at m/z 197.[7] This positive ion of the decomposition product was detected as a negative ion.

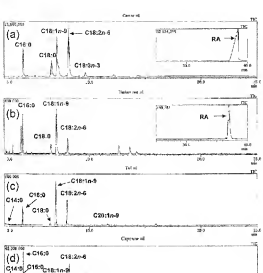

Fig. 3 Total ion chromatograms by GC-MS analysis of castor oil (a), turkey red oil (b), tall oil (c), cayenne oil (d) and tung oil (e). α-C18:3*n*-5, methyl α-eleostearate; β-C18:3*n*-5, methyl β-eleostearate.

$[M-114]^-$, in the negative mode without the lithiated adduct in this study. This character was not found in other fatty acids without a hydroxy group.

Turkey red oil. The spectrum of turkey red oil in Fig. 2(b) was similar to that of castor oil. However, a peak at *m/z* 415 was found as a negative ion of a potassium sulfated ricinoleic acid in Fig. 2(b) in the spectrum of turkey red oil because the oil is a sulfated castor oil resulting from sulfation of the hydroxy group of ricinoleic acid. This peak was very useful for discrimination between turkey red oil and castor oil. The compound detected at *m/z* 415 in the ESI-MS spectrum was not found in the total ion chromatogram (TIC) of the GC-MS analysis in Fig. 3(b).

Tall oil. In the spectrum of tall oil (Fig. 2(c)) octadecenoic acid, octadecadienoic acid, and palmitic acid, which were also detected by GC-MS analysis (Fig. 3(c)), were mainly found with peaks (30.6, 6.3 and 2.8%) at *m/z* 301, 299, and 305. These peaks at *m/z* 301, 299, and 305 were assigned to resin acids, such as an abietic acid ($C_{20}H_{30}O_2$, molecular weight (M_W) 302) of components of tall oil. The resin acids were not found in the TIC of the GC-MS analysis shown in Fig. 3(c).

Cayenne oil. In the spectrum of cayenne oil (Fig. 2(d)) the characteristic peaks were observed at *m/z* of 168, 170, 304, and 306. The peaks at *m/z* of 304 and 306 were assigned to deprotonated molecular negative ions of capsaicin (M_W 305) and dihydrocapsaicin (M_W 307), shown in Fig. 2(d), respectively. The capsaicin and dihydrocapsaicin were also detected by the GC-MS analysis of the unsaponifiables of the cayenne oil. The peaks at *m/z* 168 and 170 were assigned to fragment ions $[M-137]^-$ of the capsaicin and dihydrocapsaicin, respectively, because the EI-MS spectra of the compounds by the GC-MS analysis showed the fragment ion peak at *m/z* 137 as the base ion peak. The ESI-MS spectra of the compounds in a positive mode also showed the fragment positive ion peak at *m/z* 137 with protonated molecular positive ions, $[M+H]^+$, of capsaicin (*m/z* 306) and dihydrocapsaicin (*m/z* 308). The major fatty acids were octadecadienoic acid, octadecenoic acid, octadecatrienoic acid, and palmitic acid, which were also detected by GC-MS analysis (Fig. 3(d)).

Tung oil. A peak (approximately 31%) at *m/z* 277 in the spectrum of tung oil (Fig. 2(e)) was not assigned to linolenic acid (C18:3*n*-3, M_W 278), but rather to the α-eleostearic and β-eleostearic acids (C18:3*n*-5, M_W 278) of a main component in the oil.[25] Methyl α-eleostearate and methyl β-eleostearate were detected at 19.4 and 21.7 min of the retention time, respectively, by the GC-MS analysis shown in Fig. 3(e), as is characteristic of tung oil.[26]

Discrimination of fats and oils by fatty acid composition using ESI-MS and GC-MS analyses

Comparisons of a base peak in the ESI-MS spectra classified 28 types of the saponified vegetable oils (Table 1) into eight groups, which span the octadecenoic acid (C18:1) group of nine oils, comprising palm oil, camellia oil, macadamia nut oil, bran oil, rapeseed oil, olive oil, tea seed oil, peanut oil and cocoa butter; the octadecadienoic acid (C18:2) group of eight oils, comprising safflower oil, cotton seed oil, soybean oil, evening primrose oil, corn oil, sesame oil, sunflower oil, and grape seed oil; octadecatrienoic acid (C18:3) group of four oils, comprising perilla oil, egoma seed oil, linseed oil, and tung oil; the lauric acid (C12:0, *m/z* = 199) group of two oils, comprising coconut oil and palm kernel oil; the capsaicin group, comprising cayenne oil; the icosenoic acid (C20:1) group, comprising jojoba oil; the abietic acid group, comprising tall oil: and the ricinoleic acid group of two oils, comprising castor oil and turkey red oil. This classification of 25 types of vegetable oils, except palm oil, cayenne oil and tall oil, corroborated that by the GC-MS analysis. The basic peak of palm oil by the GC-MS analysis was not methyl oleate, but rather methyl palmitate. The detection sensitivity of the ESI-MS analysis of unsaturated fatty acids was higher than that of saturated fatty acids. Therefore, although the methyl oleate and the methyl palmitate of palm oil determined by GC-MS analysis were 40.3 and 43.1%, respectively, octadecenoic acid and palmitic acid determined by ESI-MS analysis became 63.4 and 15.2%, respectively. The basic peaks of tall oil and cayenne oil by GC-MS were assigned to the saponifiables methyl oleate and methyl linoleate, respectively. Although the ESI-MS analysis in this study detects anion compounds produced by the ESI method in both the saponifiables and the unsaponifiables, the GC-MS analysis in this study detected only the fatty acids produced by saponification. Therefore, both the GC-MS analysis and the ESI-MS analysis detected octadecenoic acid and octadecadienoic acid as the major fatty acids of tall oil and cayenne oil, respectively. Moreover, comparisons of the other peaks, except for a base peak, classified 28 types of vegetable oils into 23 groups. Both the ESI MS and GC-MS analyses had difficulty to distinguish between coconut oil and palm kernel oil, between bran oil and peanut oil, between sunflower oil and grape seed oil, and among the octadecatrienoic acid group of three oils (perilla oil, egoma oil and linseed oil).

A comparison of the base peak in the ESI-MS spectra classified 14 types of the saponified animal oils in Table 1 into five groups: the palmitic acid (C16:0) group, comprising sardine oil; the octadecenoic acid (C18:1) group of 10 oils, comprising

turtle oil, whale oil, shark liver oil, mackerel oil, cod liver oil, beef tallow, lard, mutton tallow, chicken fat and waterfowl liver oil; the icosenoic acid (C20:1) group, comprising saury oil; the docosahexanoic acid (C22:6, DHA) group, comprising cuttlefish oil; and the caproic acid (C6:0) group, comprising butter. This classification of turtle oil, whale oil, mackerel oil, sardine oil, cod liver oil, cuttlefish oil, beef tallow, mutton tallow, chicken fat and waterfowl liver oil corroborated that by the GC-MS analysis. The classification of shark liver oil, lard, and butter, however, showed discrepancies with that by the GC-MS analysis. Although the classification of saury oil was also observed between the result by the ESI-MS analysis and that by the GC-MS analysis, both analyses showed both icosenoic acid and docosenoic acid (C22:1) as major fatty acids. The basic peak of shark liver oil and lard by the GC-MS analysis was not methyl oleate, but rather methyl palmitate. These contrasting results were caused by the difference between the detection sensitivities of the ESI-MS analysis between unsaturated fatty acids and saturated ones shown in the case of palm oil. Although the GC-MS analysis in this study measured the methyl esters of fatty acids with nine (C9:0) or more carbon numbers, owing to the solvent delay, the ESI-MS analysis measured fatty acids with six (C6:0) or more carbon numbers. Therefore, the basic peak of butter by the ESI-MS analysis differed from that of the GC-MS analysis. Moreover, comparisons of the other peaks, except a base peak, classified the 14 types of animal oils into 11 groups. In both the ESI-MS analysis and the GC-MS analysis, it was difficult to distinguish shark liver oil from mackerel oil, cod liver oil from waterfowl liver oil, and lard from chicken fat.

The distinction between vegetable oils and animal oils by ESI-MS analysis was estimated by comparing the relative peak intensity of palmitoleic acid (C16:1). Macadamia nut oil and cayenne oil were exceptionally abundant in palmitoleic acid, however, and contained 27.7 and 2.5%, respectively. The palmitoleic acid of animal oils, except for butter, demonstrated more than 3.0% of the relative peak intensity, but vegetable oils, except for the two oils, demonstrated less than 1.0%. Marine animal oils (whale oil, shark liver oil, mackerel oil, sardine oil, cod liver oil, saury oil, cuttlefish oil and waterfowl liver oil) were abundant in icosenoic acid, icosapentanoic acid (C20:5, EPA), and DHA, distinguishable from the other animals. Whale oil contained more myristoleic acid (C14:1) than the other marine animal oils. These results corroborated those by GC-MS analysis.

Advantage of ESI-MS analysis compared with GC-MS analysis

All of the types of fats and oils were analyzed within one minute by ESI-MS to obtain the mass spectra corresponding to the total ion chromatogram by the GC-MS analysis of the fatty acids. The sample preparation for the ESI-MS analysis of fatty acids was reduced to two steps only: saponification and extraction. The GC or GC-MS analysis still required esterification or transesterification, however. The GC-MS analysis was much more laborious and time-consuming than the ESI-MS analysis due to the three steps required for sample preparation (saponification, extraction and esterification or transesterification) as well as the long measurement time for the elution and separation of the components of fatty acid methyl esters through the GC column. The GC-MS analysis of various types of fats and oils did not require a constant minimum measurement time because of the various retention times of fatty acid compositions. For example, the minimum measurement time required is 40 min for castor oil, turkey red oil, waterfowl liver oil, whale oil, and fish oils; 25 min for tung oil, jojoba oil, egoma seed oil, cayenne oil and tall oil; and 15 min for the other oils. As such, the ESI-MS analysis offers a time-saving alternative method for analyzing unknown fats and oils without a control sample.

Conclusion

Although the GC-MS analysis can discriminate the isomers of unsaturated fatty acids, the ESI-MS analysis summed the compounds without discrimination. The sample preparation for the ESI-MS analysis of fatty acids was reduced to two steps, saponification and extraction, compared to that of the GC or GC-MS analysis, which involves three steps, including esterification or transesterification. The ESI-MS analysis discriminated fats and oils within one minute after starting the measurement. The analytical results by the ESI-MS analysis were very useful for reducing the measurement time of the GC-MS analysis of unknown fats and oils. This is because the retention times of all fatty acids resulting from the saponified fats and oils could be identified by comparing with the spectra produced by the ESI-MS analysis. Characteristic compounds, such as resin acids and the sulfated ricinoleic acid, were not detected by GC-MS analysis, but rather by the ESI-MS analysis. Although the fatty acid composition by the ESI-MS analysis did not perfectly corroborate that by the GC-MS analysis, both the ESI-MS analysis and the GC-MS analysis could be applied to an analytical method for the discrimination of the fats and oils. Accordingly, the ESI-MS analysis of the fatty acid composition has been found to be suitable as a screening procedure for the rapid discrimination of fats and oils.

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